Validation of the production of Penicillin G

Blandine Abewe, Andrea Chakwizira, Yanyang Pei, Ragna Sandell, Steven Tran

C3: GMP & Validation + HPD

KMBF10

Instructor: Jenny Schelin

Submission date: February 26, 2024

1. Summary

The project aims to provide quality assurance throughout every stage of the penicillin production process. It includes a description of the process, a flow chart, a Hygienic Process Design (HPD), Good Manufacturing Practices (GMP) and validation, as well as evaluations of ethical considerations and the environmental impact of the process. A detailed description of the production process provides an understanding of each step involved, from the initial inoculum of penicillin- producing fungi to the downstream process and packaging stages. The process description and flow chart gives an overview as well as facilitates effective monitoring and control. The HPD helps the production facility to minimize the risk of contamination and ensure the safety of the workers, through a thorough description of the facility layout and the training of personnel and equipment used.

By adhering to the GMP standards, product quality and safety is ensured. The validation protocols demonstrate the consistency and reliability of the production process, including testing and analysis to verify the critical parameters and control measures. In this report validation is carried out on one cleaning process, one analytical method and a qualification is carried out on the fermentor as one process equipment.

Environmental and ethical evaluations reflect a commitment to sustainability and social responsibility. Measures are implemented to minimize environmental impact, such as reducing waste generation and energy consumption, while also ensuring ethical considerations are addressed such as working conditions and ethical sourcing of raw materials. The project is written following the guidelines and requirements from Eudralex and ISO standards.

2. Introduction

Penicillin was a groundbreaking discovery that changed the history of medicine. Today's world relies heavily on having access to antibiotics, and resistance to antibiotics is a major concern. We are setting up a factory in which penicillin production will be carried out. It is of utmost importance to keep the product clean but also to make sure that the environment is not contaminated and that the workers are kept safe. We lay out the process in a flow chart and a more detailed description of the production steps, and we conduct a risk assessment in which all steps of the process are analyzed. In order to ensure that people and the environment are kept safe, we make use of quality tools to qualify the production process. The first tool we make use of is Hygienic Process Design (HPD). This tool helps us to explain and identify how the production area and surrounding areas should be designed to ensure a safe process from receiving the raw materials and till the product is finished. We design a facility layout of the production site, and specify certain key areas of importance such as the equipment used, the training of personnel and cleaning routines. One cleaning in place and one manual cleaning process will be used as an example. The second tool we make use of is Good Manufacturing Process (GMP) and validation. In this section we will focus on exemplifying the qualification of one equipment, in our case the fermentor, and the validation of a cleaning in place process as well as the validation of an analytical method used to ensure the final product is up to standards.

3. Product and process description

3.1 Product description

The product we are supplying is a vial with 5 million Units of Penicillin G sodium, penicillin in powder form that the consumer mixes with solvent, e.g. water or sterile isotonic sodium chloride solution, for parenteral injection. Penicillin G sodium is an odorless white crystalline powder, and when dissolved during reconstitution it becomes colorless.

The product should have a pH range between 5.0-7.5 and is stored in 20-25 °C. The product purpose is to be an antibacterial agent injected intravenously or intramuscularly. The molecular structure is depicted below in figure 3.1. (8)

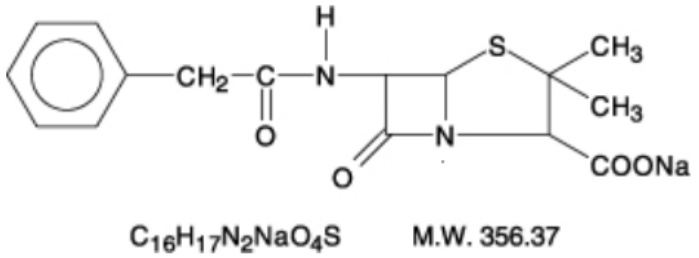


Figure 3.1: A picture of the API, the Penicillin G molecule drawn in Lewis-structure (8).

3.2 Flow chart

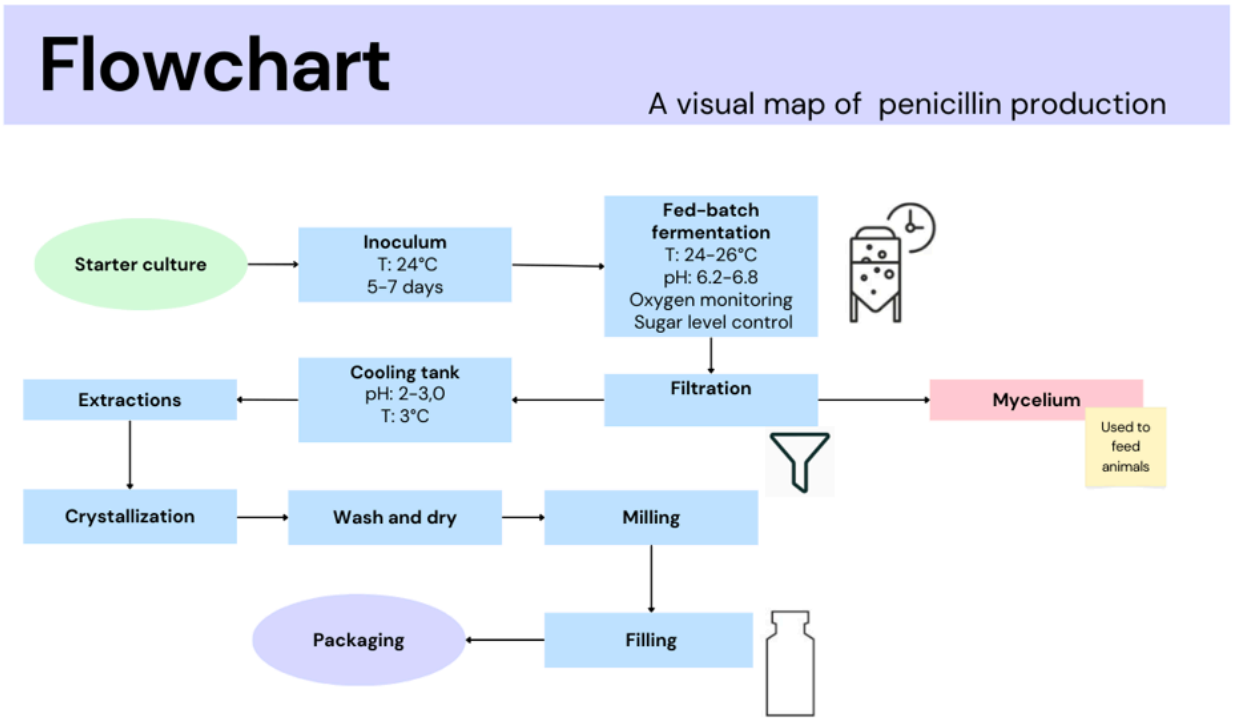


Figure 3.2: A flow chart of the penicillin production involving the production steps: Starter culture, inoculum, fed-batch fermentation, filtration, cooling tank, extractions, crystallization, wash and dry, milling, filling and packaging.

3.3 Process steps

A brief description of the process steps:

1) Starter culture

Firstly a strain is chosen based on its abilities to produce penicillin, these are extracted from frozen spores which have proven to heavily sporulate.

2) Inoculum

Now the spores will grow into mycelium. Spores are suspended in non-toxic wetting agents or water, then they are moved to a flask with wheat bran and nutrition for them to grow. Heat shock is used to make the spores rejuvenate. They are incubated for 5-7 days at 24°C for heavy sporulation. Later they are incubated in a seed tank for a shorter period of time, 24-48 h, with aeration and 24°C.

3) Fed-batch fermentation

Mycelia are moved to a fermenter tank with temperature 24-26°C and a pH around 6.2-6.8. The tank is stirred and each batch takes 3-5 days. Sugar and oxygen is monitored for the product to grow (5).

4) Filtration

The broth is separated from the biomass with a rotary vacuum filter (18). The filter pores are sized to keep other microorganisms from the product.

5) Cooling tank

The cooling tank decreases the broth to 5-10°C to reduce the reactivity of penicillin.

6) Extractions

The product is extracted from aqueous filtrate into amyl acetate in a centrifugal countercurrent extractor. The aqueous fraction is later discarded.

7) Crystallizations

Potassium acetate is added to crystallize the penicillin, in a tank, to precipitate a penicillin salt.

8) Wash and dry

The product is washed to remove impurities and residual broth, using a solvent that will not dissolve the penicillin crystals, then it is dried to remove moisture since our product is in dry format (5).

9) Milling

The crystals are broken down to a powder, to a particle size the body can absorb when reconstituted in water for injection.

10) Filling

Firstly the vials are prepared with sterilization using autoclaving. In a Grade A zone our product powder is filled in vials with an automated system. The vials have sterile closures and labeled with information about how to use the product correctly and how to store it. Here quality control tests the safety, efficiency and purity of the product.

11) Packaging

The finished product is packaged in appropriate boxes to keep the vials safe under transportation. A final batch release testing will ensure the quality and compliance of each batch, only the batches that pass this control are distributed to the consumer.

4. Results: Hygienic Process Design

4.1 Purpose of cleaning and disinfection

It is of utmost importance to clean and disinfect to keep the antibiotics inside the production facility. Antibiotics leaving the facility can endanger humans and all living kinds. The environment must be kept clean and the workers must be kept safe.

The purpose of cleaning is to reduce the amount of microorganisms in the environment that could contaminate the product, which means that the surfaces in our production should be adequately cleaned and sanitized. By removing dirt and microbial contaminants the risk of microbial growth decreases. A result of this is that the risk of having products being recalled or due to consumer illness reduces radically. For pharmaceutical products, especially injectables, the stakes are very high since any issues or recalls potentially have life-threatening consequences for consumers.

Furthermore, maintaining a clean working environment is also important for the psychosocial well-being of employees, as dirt and poor hygiene can lead to stress and allergic reactions. Having a workplace that is properly cleaned will contribute to an environment where employees can focus and work effectively. For our production it is also essential to have clean air and this is done with air locks, which purpose is to minimize the transfer of air and contaminants between different areas.

4.2. Surroundings and building

The facility or the site selection must be far away from sources of contamination, such as swamps, landfills, and any other heavy industries that can pollute the production process or can cause contamination. The selected site should have easy access to water, electricity and other utilities. Furthermore, it should be in a place where we can access easy and pure transportation without contaminating the product. The facility needs clean rooms that cannot be contaminated from internal or external factors.

4.2.1 Parameters to consider in the surroundings

- Heavily industrialization areas

The facility should be located away from heavily industrial areas to avoid air and noise pollution. Noise pollution can cause vibration which will make the building shake and dust could enter our fermenter, contaminating our fungi or spores. Air pollution, caused by different industrial activities, would lead to poor air quality which could pollute our product. At the same time the facility should be located in an area suitable for pharmaceutical industries to facilitate the ease of transportation of raw materials.

- Natural environments such as lakes, swamps, forests

Lakes and swamps are natural habitats of different microorganisms. If those microorganisms enter the production area this can contaminate material, equipment, surface and can also contaminate the fungi used to produce penicillin as well as interfering with its growth. Swamps attract pests which carry contaminants, and they can later be introduced to the production area. This can be solved by placing the facility far from the swamps or lakes. It is also important to have pharmaceutical industries away from natural environments because these environments should not be polluted by our product. Since natural environments are good breeding grounds for bacteria, it is very important to ensure that no antibiotics contaminate such environments since that would lead to a spread of antibiotics resistance.

- Landfills and waste

Landfills attract insects or pests that can be the source of contaminants and carry them in the production area which can contaminate the fungi during the production process of penicillin. Waste can also be the source of microorganisms that can contaminate the equipment, surface, raw material and product. The facility should therefore not be located close to a landfill. This is also because a landfill is a breeding ground for bacteria and should our antibiotic product pollute such an area, antibiotic resistance in microorganisms could quickly spread. It is therefore very important to have a proper waste management system in place for the production of penicillin.

- Highway and transportation

Emissions from cars can cause air pollution that can affect air quality and can contaminate production areas and personnel. Noise pollution due to too much vibration can cause buildings to shake, leading to dust in our fermenter which can contaminate our fungi or spores. Due to car movement this can bring dust that can contaminate equipment, raw material and products. Pharmaceutical industries should be located away from highways, but should at the same time have good transportation opportunities to facilitate the ease of bringing in raw materials and transporting the product.

4.2.2 Parameters to consider for the building

- Windows and ventilation

Windows are needed for the well-being of the personnel and for the design but having windows that can open may pose a risk for the hygiene in the facility. It is therefore important to ensure that the windows installed limit contaminants from the environment to enter the building or from inside the building to the environment. This is ensured by having windows that do not open and close. Fresh air entering the building will come through a ventilation system. This is to reduce the risk of airborne pollution. At the same time the air going out of the building must also be filtered to ensure that the surroundings are not contaminated by spores and antibiotics. Ventilation also provides temperature control which is important both for the wellbeing of the workers and for the production.

- Gates and doors

Doors can be used as a barrier to contain fungi or antibiotics from going outside the production area. This is done by having properly sealed doors with tight-fitting gaskets. Also setting access control to the doors will reduce cross contamination among personnel, production area and environment due to control of opening and closing doors. This will ensure that only the workers that have been trained at the required level can enter certain areas of the production. Also setting monitoring sensors will provide the real time data about the temperature and other parameters which will help to regulate the condition.

- Air locks to protect the environment

Air locks are special entry ways made of a series of interlocking doors that control movement between areas. Having higher pressure of air in some areas and lower air pressure in others, will enable us to control the airflow. By setting up this system it will help to prevent direct transfer of contaminants from the production area to the environment or between the cleanroom areas. This will also prevent personnel from being contaminated or contaminated areas and will prevent contamination of the environment. So, air lock series is needed for good hygienic practice because it allows personnel, material and raw material to transition between different areas without causing any contamination.

4.3. Interior Environment & layout design

In this part, general requirements and materials chosen for construction and layout design are described. In order to avoid glare, consideration should be given to the interaction of surface color and surface finish with the intended lighting conditions. In the case of equipment and material transfer airlocks, decontamination and cleaning procedures can impose special requirements for the selection of materials.[4]

4.3.1 Construction and assembly of an installation

1) Construction of ceiling, wall and floors

Requirements: The selection of construction technique, choice of materials and effectiveness of the design details are all intended to ensure finishes are fit for purpose by being smooth, crevice-, crack- and cavity-free, laid on even surfaces and flush with minimal steps and ledges that can create areas for contamination to collect in.

Table 4.1. Material chosen for construction of ceilings, walls and floors

	Materials	Reason
Ceiling	Aluminum	Light weight, resistance to corrosion, durable, long-lasting, easy to maintain and clean.
Wall	Coated with epoxy	Provides a smooth and glossy surface and has good performance in resisting dirt, grime, and bacteria.
Floor	Epoxy flooring	Provide a seamless surface, durable and abrasion resistant, easy to clean, simple maintenance

2) Construction of door and light

Table 4.2. Material chosen for construction of doors and lights

		Requirements
Door	Door handle	Smooth, non-snagging and easy to clean
	Door	Should be selected

	close	with minimal ledges and no uncleanable crevices or ledges.
	Door surface	Smooth, as few horizontal surface as possible
Light		Ensure visibility and facilitate thorough inspection and cleaning. Fixtures should be easy to clean and maintain.

4.3.2 Layout design:

1) Classification of rooms

The classification of rooms is divided into four grades according to its risk in operation level:

Grade A:

Aseptic, high-risk operations, in our penicillin production process, the steps performed in this area include primary packaging of product.

Grade B:

For aseptic preparation, in our penicillin production process, the steps performed in this area include the downstream process such as filtration, extraction, recovery and washing as well as drying of products

Grade C:

Cleaning of equipment, preparation of solutions, in our penicillin production process, the steps performed in this area include pre-culture of spores and preparation of solutions needed in the process and media, inoculation and fermentation can also be conducted in this area since it is performed in a closed fermentor and does not need a highly sterile environment.

Grade D:

Storage of materials, cleaning equipment, in our penicillin production process, the sterilized raw materials are stored here and outer packing is performed.

Contaminant requirement: according to cleanroom grade, the maximum limit of total particles should be controlled as the standard given in annex 1 shown in table 4.3 and 4.4.

Table 4.3. Maximum permitted total particle concentration for monitoring

Grade	Maximum limits for total particle ≥ 0.5 µm/m³		Maximum limits for total particle ≥ 5 µm/m³	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [(a)]	29 300	Not predetermined [(a)]

Table 4.4. Maximum permitted microbial contamination level during qualification

Grade	Air sample CFU/m³	Settle plates (diameter 90 mm) CFU/4 hours [a]	Contact plates (diameter 55 mm) CFU/plate
A	No growth		
B	10	5	5
C	100	50	25
D	200	100	50

2) Segregation principle

Segregation should be built to prevent cross contamination. Air flow should be established to ensure no backflow or entrainment of contamination from the less-clean zone into the cleaner zone. Pressure drop should be set between different cleanrooms. In cases where high levels of segregation are required, separative devices should be considered.

The door of the airlock should always open to the side where high pressure is maintained to prevent contamination, this can be seen in the facility layout.

3) Layout of room, flow of material and personal

The layout of the cleanroom is designed following the guidelines given in ISO 14644-4, and is shown in figure 4.1.

The warehouse is located on the ground floor (floor 0), where raw materials such as spores and nutrients for making the culture medium are stored. These materials are then transferred to the hygienic area on the first floor (floor 1) via a conveyor and stored in a grade D cleanroom after being sterilized. Cleaning equipment is also stored in this room. Once sterilized, the materials are moved to the grade C manufacturing room where the preparation of growing medium and buffer solutions takes place. In the same room, prepared materials are fed into a fermenter, where key production steps including inoculation and fermentation occur.

Once raw penicillin is produced, it is transferred to a grade B hygienic area where a series of downstream processes, including centrifugation, cooling, extraction, and recovery, are performed. Sterilization is conducted between each step to ensure the final product is 100% sterile.

The extracted and purified penicillin powder is then sterilized and transferred to a grade A cleanroom for primary packaging, also known as filling. The product is then transferred back to the grade D area via another conveyor for outer packaging.

A viewing corridor is designed to allow supervision of staff while they are in the area. Additionally, electronic access control is implemented in the grade A cleanroom to reduce the risk of unauthorized personnel entry.

Other facilities are constructed in an area outside the cleanroom to not only reduce contamination but also provide convenience. This includes a meeting room, canteen, break room, restrooms as well as a lab. Personnel airlocks (changing rooms) are situated between cleanrooms or corridors to facilitate personnel changes before entering different areas. Staff working in grade D and grade C areas are expected to enter from the grade D personnel airlocks and only pass through these two areas. Personnel working in grade B and grade A areas can enter either by passing through all the cleanrooms in other grades or through a grade B airlock on the other side. Negative pressure is required in the area where downstream processes are performed, this aims to avoid penicillin going out, and protect the environment and personnel.

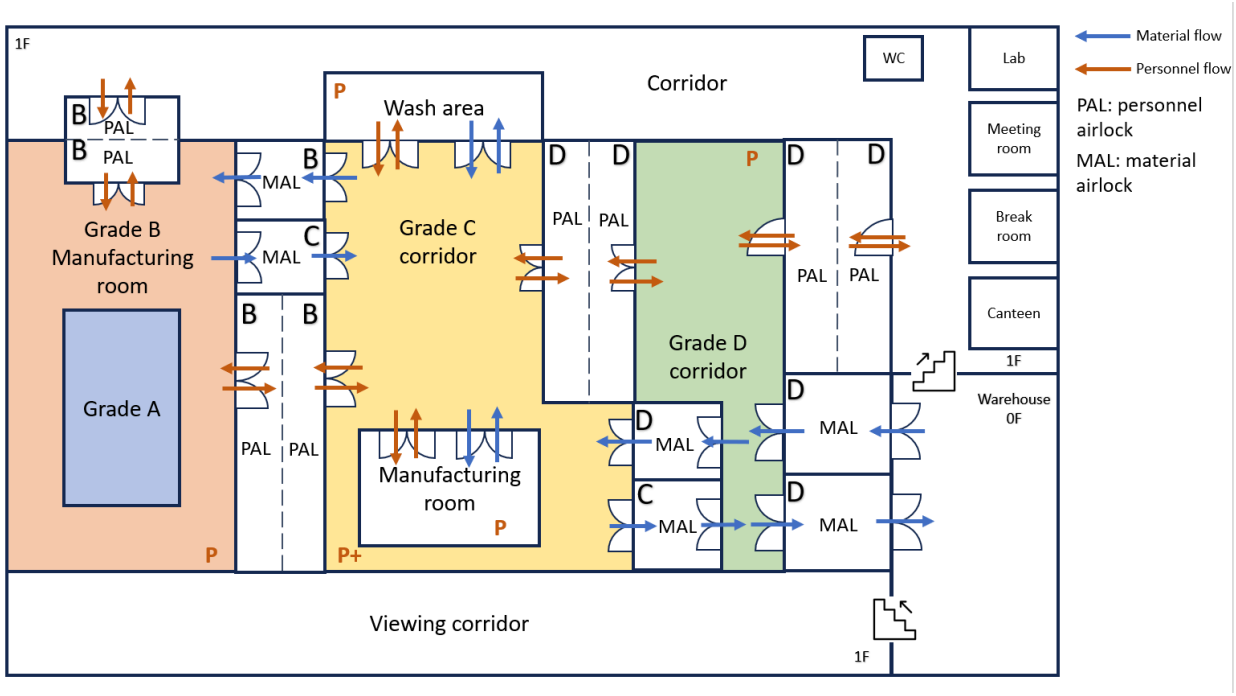


Figure 4.1. Layout design of production

Adhesive mats are installed inside the personnel airlocks to minimize the introduction of contaminants from shoes into the cleanroom. These mats must be replaced every two months to maintain their effectiveness. Lockers for personnel to store their clothes are necessary, and dirty clothes must be removed for cleaning and disinfection every three days.

Air handling units including ventilation systems including fans, air filtering equipment should be installed to ensure air exchange in rooms, but in the meanwhile, air flow should be set in an acceptable range to reduce introduction of contamination.

4.4 Hygienic process design of equipment

Hygienic process design, HPD, is embedded throughout the equipment design.The sanitary design principles are followed for best results and every step is continuously documented and validated. Five examples of key components are:

- The fermentation tank will be made of stainless steel because it has high durability and it will be designed without any sharp edges to ensure penicillin residues will not stay in the tank, thereby facilitating the cleaning and drainage. The walls will be slanted to make it difficult for dust to settle and same goes for the surface within the fermentor, it is needed to be smooth to avoid contaminants lingering or any buildup in potential cracks. There are aseptic sampling ports to enable sterile sampling, the ports are closed off from the environment via a closed circuit and have been sterilized and will therefore not contaminate the sample. The tank has its own air system to avoid air pollution from other parts of the process. CIP and SIP are incorporated into the tank (15). All requirements are listed in the User Requirements Specification below.

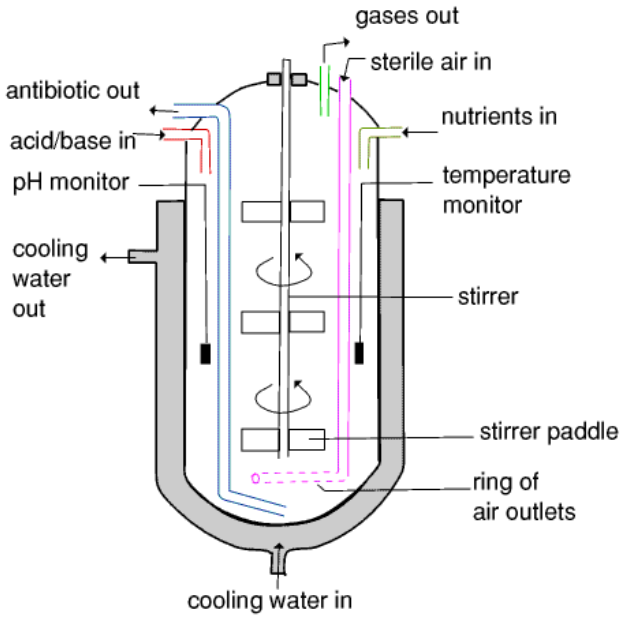


Figure 4.2: Drawing of a fermenter tank (9).

- A rotary vacuum filter made of stainless steel is used after the fermentation to harvest the penicillin broth (18). The filter has a durable material for it not to degrade over time since it is sterilized after every batch. All the broth is being transported through the filter, which has no surrounding dead space since that will hinder efficient cleaning and create bacterial growth. The pores in the filter have a pore size small enough to filter out bacteria and mycelia, around 0.1-0.45 micrometer (19).

- A pipe in stainless steel will transport the penicillin from the cooling tank to the extraction area, the material is chosen because of its inertness and durability. The surface of the pipe is smooth and the connections do not have dead legs (15)

- The valve regulates the pressure within the fermentation tank, we have a ball valve because of its rounded shape and it is relatively easy to clean and sterilize (7). It is important that no fluid escapes the valve when closed, product could be destroyed and the cleaning agents could escape the tank. The valve is self-drained which makes sure no liquid remains. It is possible for the personnel to see if the valve is open or closed.

- A centrifugal pump is used for transporting the fermentation broth from the fermentation tank to the cooling tank through the pipe and the filter. The pump is polished and has a smooth surface and it is equipped with sealing mechanisms that prevent fluids from escaping the pump. It needs to be maintained regularly through a schedule ensuring quality of the product as well as the cleaning of the pump. It is easy to disassemble and clean the different parts of the pump. Outer surface is easy to clean and leakage is easy to observe and therefore it can be dealt with directly (15).

4.4.1 Cleaning of equipment

Below is the cleaning description for the fermenter tank. This is incorporated automatically at the end after every batch using a Cleaning In Place (CIP) system. Cleaning steps for the fermentor are as follows:

1) Displace the residual carbon dioxide with air for approximately 15 minutes.
2) Rinse the fermentor with water and heat it up to 90 °C.
3) Circulate hot alkaline water, an organic solvent that is 1.5-2% and 80 °C, for 60 minutes at most. Rinse with hot water afterwards and then rinse with cold water until the fermentor has room temperature. One spray ball is located at the top of the fermentor (see figure 4.3).
4) A nitric acid solution, used as an inorganic solvent, with a concentration of 1-2% is used for 15 minutes.
5) Lastly it is rinsed with water to neutralize the drain. The water is regularly checked in the lab to guarantee no microorganisms are growing in the tank when it has been cleaned.

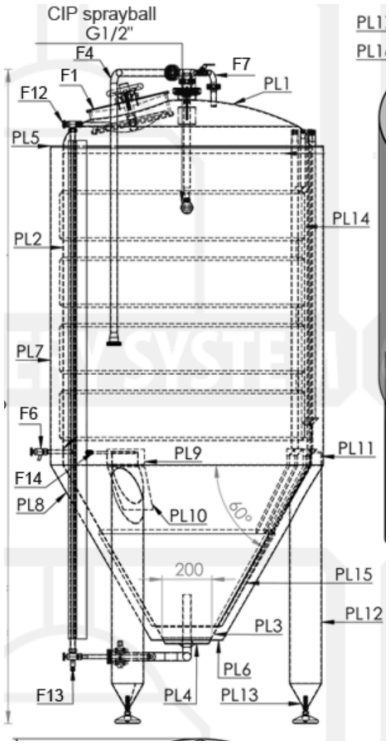


Figure 4.3: Spray ball in fermentor(17)

After every cleaning there is a sterilization in place (SIP), the sterilization is vital for the next batch to not be contaminated. The cleaning is prior because if the dirt has not been cleaned yet, the bactericide may not reach all of the bacterial surface, therefore risking not killing all of the bacteria (16).

4.5. Hygienic process design of personnel

In the pharmaceutical industry, personnel must have appropriate clothing to maintain cleanliness, minimize the contamination of product and ensure the safety standard of operator personnel from the equipment as well as product. When the personnel move from one clean room grade to another they need to change garments. Garments must be put in the laundry after wearing and a new garment must be taken and worn. The qualification of garments should consider any necessary garment testing requirements, including damage to garments that may not be identified by visual inspection alone. Garments must be chosen to limit shedding due to the operator's movement.

4.5.1. Typical clothing required for the personnel

- Hairnets and Beard mask

Personnel must wear hair nets to protect hair from falling in the product and causing contamination of the product. Personnel must wear hairnet anytime they are in an aseptic place for example in the sterile cleanroom to avoid introducing microorganism to the sample or product that can come from hair or scalp, which will contaminate the product. Hairnets will not only protect the product, but it also protects the personnel from toxic microorganisms from being exposed to their hair. In addition, personnel wearing hairnets can ensure working under regulation.

- Coverall

Personnel must put on coveralls especially in the septic place to minimize contamination of the product from any particles or dust from our clothes, microorganisms and bacteria from dead skin cells. The cleanroom overall minimizes the introduction of microorganisms from the external environment which can contaminate the product and maintain cleanness of the sterile place. Coveralls prevent personnel skin from being exposed to toxic microorganisms. Personnel coverall must not contain pockets to avoid cross- contamination between different grades or areas.

- Eyeglasses or Goggles

Eyeglasses must be worn especially for personnel safety. To minimize direct contact with eyes that can cause eye irritation, allergic reactions like eye inflammation, redness and itching. Wearing goggles protects personnel in case penicillin powder may spill or splash. Considering the production perspective, it minimizes any contamination that can be caused by bacteria from the eyes. Personnel should wear glasses or goggles when they are handling samples or final products.

- Face mask

Face masks will protect personnel and products. Personnel must wear facemasks for their safety to minimize the inhalation of the airborne particle like dust or chemical that may be available during penicillin production or in the manufacturing environment, thus minimizing the risk of respiratory illness. For the cleanroom, face masks minimize contamination that can be caused by mucus or bacteria from our nose.

- Boots/ Closed-toe shoes

During the day when the personnel walk around they might pick up some microorganisms by the shoes which could potentially contaminate the product. Therefore, personnel should wear proper shoes while entering the facility, especially in the cleanroom. The shoes also protect the personnel from harming their feet with the equipment.

- Gloves

In the handling of the product or materials during packing or any other activities that require the handling glove must be worn to protect against cross- contamination from one place to another or avoid particles or microorganism from nail/hand to contaminate the product. Gloves can be used to protect personnel from being contaminated.

4.5.2 Typical needs required for the personnel

- Somewhere to eat

In the pharma industry, personnel must have a designated area for eating to maximize hygiene and contamination control. This will avoid the presence of food in the wrong place especially in the cleanroom and cross- contamination between food and penicillin or the culture.

- Toilet

Personnel get access to the restroom to help to maintain the hygiene or sanitation practice which avoids cross-contamination. This helps regular hand washing that keeps personnel from microorganisms which result in less cross-contamination. So toilets should be integrated into rooms as much as possible.

4.5.3 Knowledge

- Enough personnel that are qualified and suitable for the work.
- Ensure they have training on the equipment and how to perform the procedure, and any technology used in the factory ensures that they have knowledge about GMP.
- Personnel with access to the cleanroom should have the training about the correct way of dressing (garments), assessment in disciplines relevant to the correct manufacture of penicillin, hygienic, specific practices on contamination control, protection of sterile product especially those who encounter grade B and A cleanroom (training should be according to the area the personnel come in contact with)
- Personnel encountering Grade A and B cleanrooms must receive training on how penicillin should be packaged and all necessary procedures to follow. They must receive training on how to use appropriate garments according to each room.
- Personnel should receive training in what action to take in the case of emergency, for example in grade C & D during the time of cleaning, they need to go out for their safety.
- Written Documentation must be available for the personnel for them to be able to work according to the needed procedures or parameters. document stating the process of how unqualified personnel should move or be in grade A and B.
- Personnel must receive training on how to work according to the legislation to comply with the GMP standard.

5. Results: Good Manufacturing Practices and validation

5.1 GMP

Good Manufacturing Practices (GMP) is a set of guidelines and regulations in place to ensure the consistent production of high-quality products. It was developed to safeguard consumer health and provide manufacturers with a framework to ensure a safe and consistent production in every step of the production process. Key aspects of GMP include having a validation master plan that outlines in detail how to carry out validations and qualifications of equipment, cleaning procedures, analytical methods,

utilities and processes, how to document every step of the process from batch records to standard operating procedures (SOPs) to validations and qualifications; and having documentation in place for how to deal with deviations in the process and changes to the process. The GMP guidelines serve as recommendations for the companies and help them comply with the regulations [1]. In the European Pharmacopoeia specific standards and monographs can be found which outline more specifically what is needed to meet the requirements of the GMP. Complying with GMP means that companies both fulfill regulatory requirements and also shows that the company is committed to delivering high quality products. A very important aspect of GMP is to carry out equipment qualifications on all equipment used, method validations on the analytical methods used, and cleaning validations. In this report we go through one example of an equipment qualification, method validation and cleaning validation, as well as specify how change control, revalidation and deviations would be dealt with.

5.2 Equipment validation

During an equipment validation, the first step is the User Requirement Specification (URS) in which all requirements needed for the equipment are specified [1]. In this report we take the fermentor as an example to perform the equipment validation on. The second part is the design qualification in which all requirements are double checked before ordering the equipment. Thirdly, when the equipment arrives we perform an installation qualification, operation qualification and performance qualification for the requirements in the URS that are specified to require those qualifications. We also discuss how deviations are handled during the equipment validation process.

5.2.1 User Requirement Specification

The scope of the system is to have a fermentor that allows for the production of penicillin from the fungi Penicillium chrysogenum. The key objective is that the fermentor needs to provide optimal conditions for microbial growth and produce the secondary metabolite penicillin. Table 5.1 outlines the User Requirement Specification for the fermentor.

Table 5.1 User Requirement Specification

No	Description			
1	Design specification	The equipment shall be suitable for the production of penicillin from the fungi Penicillium chrysogenum	I	-
2		The equipment must have inlet and outlet mechanisms	C	-
3		The equipment must have an inlet for adding acid or base to control the pH	C	-
4		The equipment must have slanting walls to ensure dust does not settle	C	
5		The equipment must have smooth walls to avoid contaminants lingering and any buildup	C	

6		The equipment must have aseptic sampling ports to enable sterile samping. The ports must be closed off from the environment with a closed circuit.	C	
7		The equipment must have its own air system to avoid air pollution from other parts of the process	C	
8		The equipment must have a spray ball inside to facilitate a cleaning in place (CIP) programme	C	
9		The equipment shall be compatible with Clean room GMP class C	I	-
10		The equipment shall operate at temperature 24-27	I	-
11		The equipment must have cooling water around it to control temperature	Q	IQ
12		The equipment shall be compatible with pH 6.2 to 6.8	I	-
13		The equipment must be closed in such a way that no penicillin produced inside will pollute the environment	C	-
14	Function specification	It shall be possible to adjust the stirring speed in the fermenter from 10-100 rpm	Q	OQ
15		The equipment must continuously log data about pH, temperature and oxygen levels. It shall be possible to trace back this information to each batch in case there is a deviation	Q	OQ
16		The equipment must have a stirring device	C	-
17		The equipment must have a monitoring system for pH, oxygen levels and temperature	Q	OQ
18	Quality and regulatory	The equipment shall be CE marked	C	-
19		The equipment must comply with European regulations for pharmaceutical equipment	C	-
20	Design Hygiene	The equipment shall be designed to minimise contamination risks	I	-
22		The product containing parts and the non-product containing parts of the equipment shall be designed to avoid residues of liquid after cleaning	C	-
24	Material and surface finish	Material certificate for all parts shall be provided	Q	IQ

25		The equipment must be made of stainless steel 316L	Q	IQ
26		Product containing gaskets, O-rings and seals shall be of a suitable material for manufacturing of pharmaceuticals	Q	IQ
27		Product containing parts of plastic shall be approved for use for pharmaceuticals	Q	IQ
28	Labeling	The equipment shall be labeled with supplier, model, serial numbers, manufacturing year and CE	Q	IQ
29	Safety, health and environment	The equipment shall be designed without sharp parts which can cause operator injury	I	-
30	Guarantee and maintenance	The guarantee must be valid for the whole system for at least 2 years	I	-
25		The equipment shall be supplied with a maintenance plan (frequency, methods and procedures)	Q	IQ
26		The equipment shall be supplied with a calibration plan (frequency, methods and procedures)	Q	IQ
27		The equipment shall be designed to facilitate ease of service, maintenance and calibration	I	-
28		The equipment shall be delivered with spare parts and tear parts for one year of normal service. The supplier shall specify a lost of the most frequent spare parts and tear parts including price, delivery cost, time for delivery and change frequency	C	-
29		If special tools are required it has to be specified by the supplier	C	-
30	Documentation	Manuals shall be written in Swedish. All other documents shall be in Swedish or English	Q	IQ
31		The documentation delivered must include an operation -, maintenance- and service manual, instrument listing, drawings, bill of materials, spare parts list, product contacting parts and CE-certificate	Q	IQ

5.2.2. Design qualification

During the design qualification, all the requirements for the fermentor listed in the URS table above (Table 5.1) will be gone through to check that the proposed design of the fermentor is suitable for its intended purpose and that it meets regulatory needs as well as process needs. This qualification will be

documented so that there is evidence that each point has been checked. After the design qualification the fermentor can be ordered from the supplier.

5.2.3 Installation qualification

During the installation qualification, the newly received fermentor will be checked to ensure that it lives up to the requirements specified in the URS and qualified in the design qualification. In the table above (Table 5.1) the last column specifies which requirements will be qualified in the installation qualification. All requirements with “IQ” in the last row will be checked during the installation qualification. If anything does not meet the requirements, it will be a deviation and the deviation protocol outlined below will be followed. During the IQ we will verify that the fermentor and its components have been installed correctly, that there is an instructions manual and a plan for the maintenance. Calibration of the instrument will also be done. Everything will be documented to ensure that the fermentor is correctly qualified.

One example of a requirement that would be tested during an IQ test is the requirement: “The equipment must be made of stainless steel 316L”. Upon receiving the fermentor we would check the supplied documentation to see that the fermentor is made of stainless steel 316L as required. If it is not, it must be made of stainless steel of an even higher quality. If the fermentor is made of stainless steel of a lower quality it would not pass the installation qualification and we would have to contact the supplier and ask them to take back the fermentor.

5.2.4 Deviations

Any deviations from the written procedures shall be recorded and justified. Both corrective action and preventive action shall be taken to eliminate the cause of the detected deviation, unless the deviation is acceptable. An acceptable deviation may be the type of material that the fermentor is made of, and it may still be accepted that there is a deviation from the URS if it still meets the requirements.

5.2.5 Operational qualification

During the operational qualification (OQ) it is important to ensure that the fermentor is operated as designed. The OQ may be combined with the IQ. During the OQ we would check and test the upper and lower operating limits, which can also be referred to as the “worst case” conditions. During the OQ, standard operating procedures (SOPs) would be written and cleaning procedures would be designed and qualified. Personnel would be trained on using the equipment and maintenance procedures would be established. The URS table above (Table 5.1) has specified in the last column with “OQ” which requirements would be looked at during the operational qualification.

An example of a requirement that would be tested during an OQ test is the requirement: “It shall be possible to adjust the stirring speed in the fermenter from 10 Hz to 50 Hz continuously with an accuracy of 5%”. This would be measured in a test. The stirrer would be started and the rotation speed would be noted down. It is important to let it stir for about 2 minutes before starting measurements.

5.2.6 Performance qualification

During the performance qualification (PQ) the process would be tested using production materials or qualified substituents. Here we would use the fermentor to grow penicillin from the spores from Penicillium chrysogenum. During the PQ we would also establish the required sampling procedures used to test the process. The sampling must allow the tests to cover the operating range of the intended process. The goal of the PQ is to establish that the process consistently produces the outcome according to the set specifications. Test with a real product to make sure it works as expected. Everywhere where you have OQ there will be a PQ. Do it a number of times in a row successfully in order for the PQ to be successful. Maybe five times.

5.3. Method validation

A specification has been written for the penicillin product, Penicillin G. A specification is an agreement between the authorities and the producer of pharmaceuticals stating that every batch must live up to the specified analytical controls. Components to look into in a specification are identity, visual inspection, content, impurities, uniformity of content.[1]

The goal of validating the method is to ensure that we are producing the same quality products at all times. The method will be carried out five times or more to ensure that it is consistent and reliable. After production has started, the method will be validated yearly. During the method validation we want to ensure that the method used to test the finished product is consistently reliable. We must verify the purity, stability and integrity of the final product. We must ensure that the detection limits and the quantification limits meet the requirements. Prior to the method validation we can assume that the instrument used in testing the final products, the High-Performance Liquid Chromatography (HPLC), has already been qualified according to the IQ, OQ and PQ. Thus, what is left to focus on is that the method, used for the testing, is validated.

The instrument we use to perform the method is a Shimadzu LC-2010HT which is connected to a computer on which our chromatographic method is set. We use a UV detector. The column used is a C-18 (25 cm x 4 mm, 5 micron). The mobile phase consists of water, acetonitrile and glacial acetic acid 500:500:5.75. The flow is set to 1 ml/min and the wavelength to 254 nm. The injected volume is 10 **µ**l. We follow the method set by Baghel et al. [11]). We use a penicillin G reference substance for comparison, which is weighed out and dissolved in the mobile phase as a stock solution. In order to validate the method we must set acceptance criteria for each parameter. These are discussed below.

5.3.1 The seven parameters to test during method validation

During the method validation the following seven parameters will be tested:

1) Detection limit
2) Quantification limit
3) Accuracy
4) Specificity
5) Precision

6) Linearity and Range
7) Robustness

1) Detection limits
We must have an appropriate detection limit of the penicillin. It can also be important to establish a method that has an appropriate detection limit for known impurities. The method we develop must be strong enough to detect the analyte of interest which will be both the penicillin we produce and any known impurities. The limit of detection (LOD) is accepted when S/N ratio >3 in analytical chemistry. Detection limits are determined by making a dilution series of the penicillin and of the known impurities of interest and then performing HPLC on these samples. The peaks of the analytes of interest are then integrated to find the area. The same is done for the noise peaks. The signal to noise ratio is then determined. For the dilution concentration that has a signal to noise ratio lower than 3, that will be the LOD.

2) Quantification limits
We must have an appropriate quantification limit of the penicillin. It can also be important to establish a method that has an appropriate quantification limit for known impurities. LOQ is determined in the same manner as the LOD above. The LOQ is found at 3 standard deviations above the LOD.

3) Accuracy
Our instrument must have a high enough accuracy that we can trust the results. Accuracy refers to the distance between the average measured value and the reference value. To determine the accuracy we have to compare with an established analytical reference. We use a t-test or regression to compare the measurements we obtain to the reference value. The accuracy must be within a narrow range to ensure that the vials all contain the same amount of penicillin. We have determined that it is acceptable for the signal to be off by 2 %.

4) Specificity
Specificity refers to the ability of the method to specifically determine an analyte in a mix of many other components. Specificity has to do with the method being selective enough. This will be validated by using standard solutions and spiked samples.

5) Precision
Precision refers to the spread of the data. Measurements must be close to each other to have precision. In order to trust the method it must produce precise results. If the uncertainty is too high the results cannot be trusted.

6) Linearity and range
Linearity refers to the ability of the method to give results that are proportional to the amount of concentration in the sample. We investigate this by looking into the graphs to see the linear fit. We can also compare the residuals versus the concentration to find out if it is random, what we want, or if there is a clear correlation that is not linear. The range refers to the range at which the linear fit is accurate. The method must always only operate in the range in which there is a linear fit. Outside the linear fit the

method is not valid. When using linear fit, the correlation coefficient must be reported and should be as close to one as possible. We have set the acceptance criteria for the r^2 value to be between 0.995 and 1.

7) Robustness
The method needs to be robust enough that small changes in the method, such as the flow rate, pH or temperature, or even the person carrying out the method, does not change the outcome of the method. This means we would need to carry out at different times of the day and by at least three different personnel. We would also make deliberate minor changes in parameters such as flow rate and pH and then calculate the percentage deviation.

5.3.2 Stability investigations

Since penicillin decomposes over time, it is important to carry out a stability investigation in which the stability or the decomposition of the product is determined. During the pre-formulation stage the stability of the active pharmaceutical ingredient is tested. During the formulation of the product the shelf life is investigated. The product is tested both under normal storage conditions and under accelerated storage conditions in which the temperature and humidity is increased. Specific stressors are also tested such as the photostability of the product as well as the behavior of the product during freeze-thaw tests. The product is tested in its final packaging and the three first batches of the final product will be placed in a stability investigation. Before launching the product we will have carried out a stability investigation which ensures that our product maintains the same quality throughout its shelf life.

5.3.3 Deviations

Any deviations from the written procedures shall be recorded and justified. Both corrective action and preventive action shall be taken to eliminate the cause of the detected deviation. Batches may need to be recalled because of the deviation. Recalls can be either class I, II or III or it may be a market withdrawal.

5.4. Cleaning validation

Cleaning validation is a process that makes sure that equipment and facilities are properly cleaned before use in drug or medical device production. In this cleaning validation we focus on validating the CIP programme for the fermentor described above. The CIP programme to clean the fermentor is described in section 4.4. It makes use of a spray ball located inside the fermentor to spray both water and chemicals. The cleaning validation ensures that the CIP programme can be trusted to clean the fermentor effectively and consistently. During our cleaning validation, the process is run and the CIP programme and after each cycle a swabbing test will be performed. [1]

The cleaning validation should be done with a swabbing test on the part that is particularly challenging to clean and extra susceptible to product buildup, those parts are called hot spots. Examples of hot spots could be the bend in the fermentation tank furthest away from the feed inlet of the CIP, because then the pressure and temperature might not be the same as close to the feed inlet. For this report the hot spot identified is on top of the fed-batch fermentation tank because when using equipment such as a spray ball the water and chemicals used during the CIP will be pulled down by gravity, potentially leaving the bottom comparatively cleaner than the top.The cleaning validation for this part should be done with a

swabbing test after CIP has taken place and the surface of the equipment is dry.

The purpose of the swabbing is to make sure that the cleaning procedure was effective and to get rid of all the remains of product from batches before, while simultaneously not leaving any cleaning residues. The swab head will be of a polyester material since it has low fiber release properties and is a common material used in pharmaceutical industry for cleaning validation. [12]

The swab should be rotated to try to get as much residue as possible and to not collect all samples in one area on the swab. After swabbing the swabs are labeled and put in a clean container and stored for further analysis. The container used will be a plastic centrifuge vial.

After swabbing, the surface will be inspected when dry to see if it is visually clean. Additionally the swab sample will be analyzed with HPLC and must not reach more than 10 ppm in order to pass the cleaning validation. In order to know if our method is clean, five consecutive applications of the cleaning procedure should be done and be successful in order to prove that the method is validated. [13]

Another cleaning tool that will be implemented is a water rinse sample. Rinse sampling involves using the final rinse water from cleaning production equipment as a sample for further analyzes such as HPLC. With this method it is possible to determine the quantity of residual substances present on the equipment surfaces.

5.5 Exemplify a change control

Changes in the pharma industry will happen over time, when new technologies arise this is inevitable. Examples of changes are modifications of computer systems, equipment and formulations.

When a change occurs there will be a risk analysis to determine what actions need to be changed because of that change. A team of experts from different areas will evaluate the change to make sure that the change is technically justified. (the team could be in different areas such as quality, pharmaceutical development, regulatory affairs)

To demonstrate, when a change in formulation occurs in the product this is considered as a major change. (According to EudraLex Volume 4 GMP there must be written procedures for the change protocol.) There are different steps associated with this, a widespread model for changes is as the following:

1. Initiate Change Request
2. Perform Impact Assessment
3. Review Change Request
4. Approve Change Request Plan
5. Implement Change
6. Provide Training (As Applicable)
7. Monitor Change Effectiveness [14]

The first step Initiate Change Request the step is about documenting the change in the formulation, since changes in formulation is considered a major change it will require more documentation than for instance to replace a pump with a new one where both have the same design which is considered a minor change.

The second step Perform Impact Assessment is about assessing the effect of the change of the products safety, quality, and the rules and standards.

The third step Review Change Request is where qualified personnel examine the change request.

In the fourth step called Approve Change Request Plan the person that is responsible passes the change of formulation or rejects it.

If the change in formulation is accepted the implement change involves that the company continues with executing the formulation change, which may involve updating standard operating procedures, adjusting manufacturing processes, selecting new raw materials, and updating standard operating procedures.

After that the assignment is to Provide training for personnel about the change in formulation. The training could for instance be educating the personnel on any potential danger with the new formulation, or how to operate a new equipment.

When the change is employed, it is time to see the impact of it and an evaluation of the change will be done, this is called Monitor Change Effectiveness. Under this evaluation deviations from the expected result will be noted.

5.6 Exemplify a re-validation

Revalidation is needed when a change has been made for instance in the manufacturing. The purpose is to make sure that the product produced is safe and still of high quality.

An example of re-validation in our process would be if a pump would start malfunctioning, a consequence of this would be to replace it with another pump. In this example the new pump would not have the same characteristics as the former pump therefore the same model mentioned for the change validation will be done on the pump. If the results differ from the former pump and the current, we will perform a revalidation of the pump and that means a new equipment qualification of the pump will be performed.

5.7 Demonstrate a handling of deviation

A deviation is a divergence from the established protocol or process. It can happen during any of the process steps, from development to labeling and packaging and storing of the finished product. If there is a deviation in the process, we will document it in a deviation report. Here we will state what the deviation is as well as the proposed course of action. Corrective and Preventive Action (CAPA) is a method to address and resolve deviations. We will have protocols in place for how to deal with different levels of deviations. The course of action depends on the deviation. In some cases the deviation is acceptable and the process can continue. In other cases the deviation is not acceptable and measures have to be taken. If

the deviation is found after the specific batch of penicillin has been sent to market we will have to recall the batch.

5.8. Define the type of validation performed

It is a prospective validation, done before the production starts on a new set up. Another type is a concurrent validation, but that is not what is performed here. In this report the type of validation performed is a validation master plan following GMP. The factory is set up according to the tool called Hygienic Process Design (HPD), which helps to set up a facility in which safety is thought about in every step of the process. GMP is the regulation to follow and the ISO standards help us to set up good protocols for validating methods for cleaning and analytical methods, as well as to qualify the equipment used.

6. Effects /environmental impacts

The main environmental effect of penicillin production factory would be in the following aspects:

1) Water pollution: in the production process of penicillin, different aqueous materials such as the raw materials and cleaning agents are used and poured as waste, which might pollute the environment and further cause damage to human health.
2) Air pollution: in the production of penicillin, there's spores and some volatile components might also cause effects to air quality and human health.
3) Solid chemical / biological pollution: in the production process of penicillin, solid waste including mycelium removed from the fermentation process and incubation medium can cause serious environmental hazards if not being properly handled. They should be incinerated.

Wastewater samples should be taken every day and checked to see if it satisfies discharge requirement, test record should be saved in file for future tracing. And air quality sensors should be used to test air quality around the plant every week. Effective waste treatment such as application of filtration film is essential prior to waste discharge, alongside the installation of an adequate air ventilation system to align with the criteria delineated in the ISO 14001 environmental management standard.

7. Ethical considerations

Antibiotic resistance is a major concern of today. According to the Lancet, in 2019, the death of 4.95 million people were associated with antimicrobial resistance. [14] Furthermore the Public Health Agency in Sweden estimates that 70 954 people in Sweden will be affected by resistant bacteria by 2050 which is more than 55 000 people in comparison to 2018. This can be seen in figure 7.1. The ethical dilemma lies in balancing the necessity of helping people and to take action to reduce the risk of developing antibiotic resistance.

Using antibiotics more frequently increases the likelihood of bacteria developing resistance. When bacteria survive antibiotic treatment, they have the chance to grow and spread, especially in environments where other bacteria have been eliminated.

It is crucial to find a middle ground between making sure antibiotics are available to those who need them while also ensuring they are used responsibly to reduce overuse of antibiotics, which can lead to the rise of antimicrobial resistance.

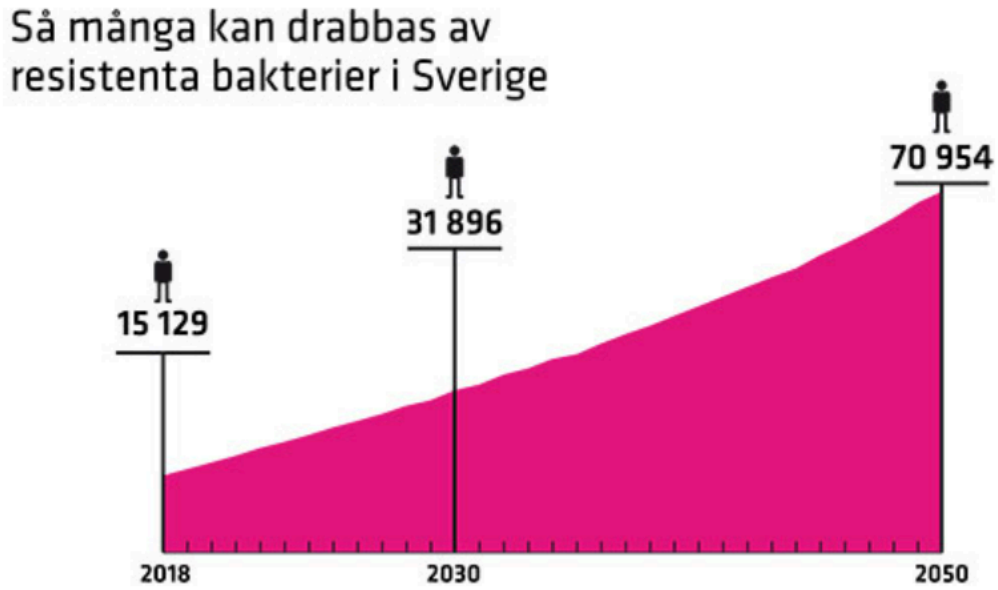


Figure 7.1, shows the number of reported cases of people being carriers of antibiotic resistant bacteria 2018 and estimated amount for 2030 and 2050.

An important ethical aspect to consider is where the raw materials come from. We must ensure that the strains used and nutrients used, and even the equipment material that the factory is set up from, is sourced ethically. This means considering both the environmental impact and the labour practices of the suppliers of material. Not only is it important to consider where the raw materials come from, it is also extremely important to ensure that the waste produced in the process is reduced. We must also make sure that as many resources as possible are conserved. We must implement the green chemistry principles wherever possible.

Another important ethical aspect to consider is social responsibility. We must ensure fair labour practices, and since the process is carried out in Sweden, we already have strong labour rights in place. A very important point when dealing with antibiotics is to ensure the safety of the workers. This is done through the validation management system and the facility lay out. It is of utmost importance that the workers do not develop antibiotic resistance, since that is a major issue, as discussed above.

In addition an important ethical aspect is the transparency of the production process. We must ensure that we have transparent communication with stakeholders, both employees, suppliers, customers and the public. We must also ensure that the products are labeled clearly and accurately and that it provides information about ethical considerations and sustainability if possible. Our company will publish relevant information about environmental impacts and what is being done to ensure a sustainable production. Our

company will make sure to conduct regular ethical compliance audits to ensure that we are following the necessary guidelines and regulations and to ensure that we continuously improve our process.

One way that our company tries to deal with the issue of antibiotics resistance is by educating people about antibiotics. It is essential that everyone has an understanding that antibiotics only work against bacteria and not viruses. Our company puts resources into campaigns to limit the selling of over the counter antibiotics. Furthermore we have discussions with veterinarians about the use of antibiotics in farming and rearing of animals. We encourage that antibiotics are given only as treatment in animal rearing and not as a prevention of infections. We are committed to encourage the responsible use of antibiotics so that antibiotic resistance will not increase and become a task too challenging for future generations.

8. Reflections and concluding remarks

We have worked well together as a group. We have had weekly meetings between us and the supervisor and we have all put good effort into the project. We have been supportive of each other and even started a tradition of someone bringing fika to our meetings and writing sessions. It has been a challenge to take in the wealth of information that exists on the topic. We were positively surprised to see just how much work goes into ensuring that the products we use are safe. It feels comforting to know that all companies must comply with these regulations, and that we have strong regulations in place. We had not thought about just how much trust we put in companies when we buy both food and pharmaceuticals. Initially it scared us to think that there are such high risks associated with the intake of food and pharmaceuticals. In our future careers as engineers we will bring with us the knowledge and understanding of food safety and pharmaceuticals regulations and what a big job it is to quality assure a process.

9. Acknowledgements

We acknowledge the use of ChatGPT to help with formulations of sentences and getting ideas for how to structure certain parts, but everything is written by us and nothing is copy pasted. We have not used ChatGPT as a source of scientific information. We acknowledge the use of ChatPDF to extract relevant information from scientific literature.

10. References

1. EudraLex. 2015. Annex 15: Qualification and Validation. EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use.2015. https://drive.google.com/drive/folders/1XJSET8lCloWEqNbe4zfxghx77cYhy12q (accessed 2024-01-20)

2. EudraLex. 2022. Annex 1:Manufacture of Sterile Medicinal Products. The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for MedicinalProductsforHumanandVeterinary Use. 2022.https: //health.ec. Europa. eu/system/ files/ 2022-08/20220825_ gmp-an1_en_0(accessed 2024-01-20).

3. EudraLex. 2022. Annex 15:Pharmaceutical legislation on notice to applicants and regulatory guidelines for medicinal products for human use.Volume 4 EU Guidelines for Good Manufacturing Practice for MedicinalProductsforHumanandVeterinary Use. 2022. Https: //health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-2_en (accessed 2024-01-20).

4. ISO standard. ISO 14644-4:2022.Cleanrooms and associated controlled environments — Part 4: Design, construction and startup.swedish institute for standard.https://www.sis.se/en/produkter/environment-health-protection-safety/air-quality/cleanrooms-and-as ociated-controlled-environments/iso-14644-42022/.

5. Gaurab Karki, 2018-12-17, Penicillin production by fermentation biotechnology, Online Biology Notes, https://www.onlinebiologynotes.com/penicillin-production-commercially-by-fermentation-biotechnology/#:~:text=For%20inoculum%20preparation%2C%20spore%20from,5%2D7%20days%20at%2024C (Accessed 2024-02-25)

6. Murray, Christopher J L et al. 2022. The Lancet. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. https://www.thelancet.com/journals/ lancet/article/ PIIS0140-6736(21)02724-0/fulltext#%20 (Accessed 2024-02-15)]

7. Ball valves, Industrial Quick Search, 2024, https://www.iqsdirectory.com/articles/ball-valve.html (Accessed 2024-02-25)

8. Sandoz Inc, 2020, DailyMed, Penicillin G sodium- penicillin g sodium injection, powder, for solution, https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=23b6d4a3-b273-4e10-9da9-1376933fdbdf&type=display#:~:text=Penicillin%20G%20sodium%20for%20Injection%2C%20USP%20is%20supplied%20in%20vials,million%20units%20of%20penicillin%20G. (Accessed 2024-02-25)

9. Richard Steane, Production of Penicillin in Industrial Fermenters (Bioreactors), Biotopics, https://www.biotopics.co.uk/microbes/penici.html (Accessed 2024-02-25)

10. Diaz-Bao et al. 2015. Fast HPLC-MS/MS Method for Determining Penicillin Antibiotics in Infant Formulas Using Molecularly Imprinted Solid-Phase Extraction. *J Analytical Methods Chemistry*

11. Baghel et al. 2009. Analytical method validation for tablet of phenoxymethyl penicillin potassium by RP-HPLC method. *Journal of Chemical and Pharmaceutical Research*, 2009, 1(1): 271-275

12. Kalelkar, Sandeep. CEMAG Care. Why The Swab Matters in Cleaning Validation. *CEMAG Care.* https://www.texwipe.com/Content/Images/uploaded/documents/Technical-Data/Why_Swabs_Matter.pdf. (Accessed 2024-02-25)

13. Creaner B, Gerry. GetReskilled. GetReskilled. What is Cleaning Validation in the Pharmaceutical Industry? *GetReskilled*. https://www.getreskilled.com/validation/cleaning-validation/#1 (Accessed 2024-02-25)

14. De Lucca Caetano. Bruna. SimplerQMS. Change Control in Pharma: Requirements and Process *SimplerQMS*. https://www.simplerqms.com/change-control-pharmaceutical/ (Accessed 2024-02-25)

15. Matts Ramstorp, 2024, Hygienic Process Design. Clean-room Techniques and Filters, Lecture notes KMBF10 2024-01-25 (Accessed 2024-02-25)

16. How to clean a fermentation tank, 2022-09-19, Micet Craft, https://www.micetcraft.com/how-to-clean-a-fermentation-tank/ (Accessed 2024-02-25)

17. CCT Cylindrically-conical tanks - Cone fermentors, Czech brewery system, https://www.czechminibreweries.com/production/brewery-components/cold-block/cylindrical-conical-fermentation-tanks/ (Accessed 2024-02-25)

18. S.Z.Li et al. 2004, Application of ultrafiltration to improve the extraction of antibiotic, https://www.sciencedirect.com/science/article/abs/pii/S1383586603001850 (Accessed 2024-02-25)

19. Nakai, 2020, Size matters: Ultra small and Filterable Microorganisms in th Environment https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7308576/ (Accessed 2024-02-25)

11. Appendix

Table 11.1 Scale for the risk assessment

Severity		Probability	
Very high	8	Very probable	4
High	6	Common	3
Medium	4	Unlikely	2
Low	2	Rare	1

Risk tool FMEA

11.1 Risk assessment

No.	Process Step	No.	Hazard and effect	Severity / Probability		Sum	Preventative measure
1	Delivery of raw materials	1.1	Occurance of metal	2	1	2	Metal detection
		1.2	Occurrence of unwanted bacteria	2	2	4	Require microbial analysis from vendors that sell it
		1.3	Genetic instability of spores	2	2	4	Take sample of spores and check that it produces what we want Require microbial analysis from vendors that sell it
2	Pre-culture	2.1	Contamination of unwanted spores	4	2	8	Subculturing and microscopic observation
3	Inoculum	3.1	Physical contamination	6	2	12	Aseptic technique, sterile equipment and training of personnel. Garments and hairnets worn.
4	Fed-batch fermentation	4.1	Temperature	2	1	2	Temperature monitoring
		4.2	Oxygen	2	1	2	Oxygen level monitoring
		4.3	pH	2	1	2	pH monitoring
		4.4	Stirrer is stuck	2	2	4	Perform equipment checking and remove mycelium regularly
5	Filtration	5.1	Mycelium is not properly removed	4	1	4	Use the right filter rating
6	Cooling	6.1	Cooling temperature	2	1	2	Monitor the temperature

7	Extraction	7.1	Nutrients and salts and precursor molecule (interference) are not properly removed	8	2	16	Take a sample and check the purity eg. in an HPLC/GC
8	Recovery	8.1	pH of the sodium hydroxide?	2	1	2	pH monitoring
9	Washing and drying	9.1	Drying temperature	2	1	2	Temperature monitoring
		9.2	Drying time	2	1	2	
10	Milling	10.1	Moisture in the product	6	1	6	Dry air of a higher temperature to ensure no moisture
11	Final product testing	11.1	Metal in the product	8	1	8	Metal detection
		11.2	Product not pure	8	2	16	HPLC
12	Packaging	12.1	Product not properly sealed	6	2	12	Seal integrity check

11.2 Standard Operating Procedure

Instructions for handling sampling for HPLC analysis

1) Purpose/scope

This SOP explains how to dissolve penicillin powder in solvent to analyze during the HPLC analysis to ensure that the product is pure and contains the amount of penicillin required.

2) Responsibility

Dissolving penicillin powder is the responsibility of the lab technician in the team of quality control. The head of the team is responsible for making sure that everyone in the team knows how to dissolve the penicillin powder in solvent for HPLC analysis.

3) Frequency

Samples will be taken from every batch produced so it will be a weekly task to dissolve the penicillin powder and perform the HPLC analysis.

4) Performance/instructions

a) Equipment

- Acetonitrile as a solvent
- Cylinder for measuring right amount of solvent
- Beaker for dissolving in
- Vortex for stirring
- Scale for weighing penicillin powder

b) Sampling

i) Take the vials that have been selected for sampling
ii) Using the scale measure 300 mg of powder from each vial
iii) Pour the powder in a beaker, one beaker for each sample vial
iv) Pour 5 ml acetonitrile in the beakers to dissolve the penicillin powder in the acetonitrile
v) Make sure everything is completely dissolved by using the vortex
v) Transfer the the dissolved penicillin into HPLC vials

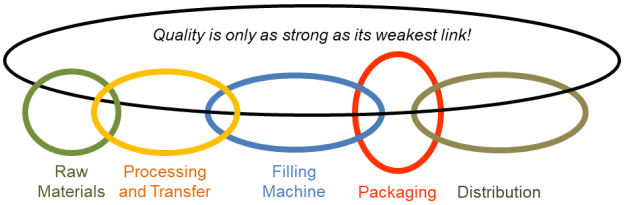
5) Documentation

Note down the exact amount of penicillin powder measured, sample ID and your signature.

6) References

Baghel et al. 2009. Analytical method validation for tablet of phenoxymethyl penicillin potassium by RP-HPLC method. *Journal of Chemical and Pharmaceutical Research*, 2009, 1(1): 271-275

11.3 Protocol from first group meeting



Quality and Product Safety [KMBF10}
Project Group Contract

Group Members:
Steven Tran, Yanyang Pei, Blandine Abewe, Ragna Sandell, Andrea Chakwizira

Your process:
Penicillin

1. Get organized and plan your project:

Create a timeline for your project:

own deadline for draft: 9:00/15/02

Plan and schedule group meetings:
Weekly meeting Monday 9:45

Distribute the work of the project:
Divide it into
1) Hygienic process design (Yanyang, Blandine, Ragna)
2) GMP and validation (Steven, Andrea)

2. Discuss and formalize the expectations of the group:

Discuss the **ambition level** between group members.

- Do you all aim for the highest score or are you satisfied if you pass?

  All agreed on do the best on report and presentation, not necessarily to get the highest score
- Are you all on the same or different levels?
  We have discussed and come up with that doing the work properly will give a satisfying outcome.
- How to handle different ambition levels in the group?
  Communication and doing our best.

Goal and ambition with the project: As a group, we agree to....

To do our best. Have weekly meetings, (have weekly supervisor meetings if possible). We agree to be responsible for our part of the project and keep the deadlines.

Discuss how you will keep up a **continuous and good collaboration and communication** in the group.

Collaboration and Communication: As a group, we agree to...

Answer messages and confirm meeting times. We agree to show up on time for the meetings. Also have a willingness to discuss potential concerns. Every meeting check in on everyone in the group how it's going with their part and if they are stuck on anything.

Perform your first **risk assessment**!

- What can go wrong/fail in the project?
  If someone isn't doing their part and not letting the others know. Can redistribute roles then.
- How to act if something does not work as planned?
  Talk to the person, not about the person with others. If we see no change we will discuss with the supervisor. If someone have trouble finish on time (tell others as soon as possible!!!), give sympathy, and give more time, and possible redistribute the work (but still so that it is a fair workload), but be strict to deadline. If don't know how to do, talk during weekly meeting (or any other time) and ask for help.

In case something goes wrong: As a group, we agree to....
First talk about the concern, thereafter try to resolve the issue and find possible solutions, if it's not possible to solve within group then go for supervisor

---

By submitting this group contract in Canvas latest on Friday 26th at midnight (24:00), you unanimously agree with the content of the contract.

Date: 2024-01-24

Group Member's Name	Group Member's Signature
Blandine Abewe	
Andrea Chakwizira	

Yanyang Pei	
Ragna Sandell	
Steven Tran	